# EXHIBIT B (PART 2 OF 2)

**Table VIII**

*Amylose Percentage of Starch from 16 Maize Genotypes Determined Following Sepharose 2B-CL Column Chromatography and the Peak Fraction's Absorbance Maxima and Extinction Coefficients (E) of the Polysaccharide–Iodine Complex*[a]

| Genotype | Amylose, % | Peak fraction (tube no.) | Maximum absorbance, nm | E at 615 nm |
|---|---|---|---|---|
| *Normal* | 29 | 14 | 510–540 | 22 |
| | | 31 | 640 | 121 |
| *wx* | 0 | 14 | 470–480 | 24 |
| *ae* | 33 | 13 | 540–550 | 43 |
| | | 33 | 600 | 92 |
| *su* | 65 | 13 | 480–530 | 28 |
| | | 28 | 640 | 97 |
| *du* | 55 | 14 | 480–500 | 35 |
| | | 28 | 640 | 104 |
| *ae wx* | 0 | 13 | 530–540 | 49 |
| | | 21 | 530–540 | 39 |
| *ae su* | 28 | 13 | 540–550 | 48 |
| | | 21 | 540–560 | 51 |
| | | 29 | 640 | 95 |
| *ae du* | 47 | 14 | 530–540 | 40 |
| | | 31 | 640 | 90 |
| *du su* | 70 | 14 | 540–570 | 47 |
| | | 29 | 640 | 92 |
| *du wx* | 0 | 14 | 470–480 | 20 |
| *su wx* | 0 | 14 | 495–505 | 32 |
| *ae du su* | 31 | 14 | 530–550 | 49 |
| | | 23 | 540–560 | 40 |
| | | 31 | 640 | 83 |
| *ae du wx* | 0 | 14 | 460–500 | 33 |
| | | 24 | 460–500 | 22 |
| *ae su wx* | 0 | 13 | 540–550 | 43 |
| | | 21 | 530–540 | 34 |
| *du su wx* | 0 | 14 | 460–480 | 17 |
| | | 25 | 450–470 | 14 |
| *ae du su wx* | 0 | 13 | <400 | 25 |
| | | 24 | 450–470 | 19 |
| *su* phytoglycogen | 0 | 13 | ≤400 | 16 |
| | | 22 | ≤400 | 6 |
| Amylose–amylopectin 1:1 mixture | 51 | 14 | 470–530 | 31 |
| | | 31 | 640 | 103 |

[a] Maize genotypes converted to the IA5125 sweet corn inbred background. Date adapted from Yeh and co-workers (*71*).

intensity of birefringence of *normal* granules, but has little effect on that of *wx* granules (*71*). The BEPT of *wx* granules is similar to *normal*, and both have A-type x-ray diffraction patterns (Table V).

Kernels of *wx* have the major and minor (type I) developmental gradients characteristic of *normal* kernels (*26, 27*). Saussy (*26*) observed the presence of occasional starch granules surrounded with phytoglycogen; however, this was due to the sweet corn background used in her study and not to the *wx* gene itself. Simple, spherical starch granules are initially produced in *wx* kernels, and these increase in size and, in many cells, become irregular in shape due to extensive cell packing (*26, 27*). Boyer and co-workers (*27*) reported that all starch granules were initiated at essentially the same time and that there was no evidence of additional granules (secondary granule initiation) being initiated later in development. Saussy (*26*) reported secondary granule initiation in *wx* as well as in *normal* and most other mutant genotypes. Boyer and co-workers (*27*) used *wx* in a dent background, while Saussy (*26*) used a sweet corn background.

As noted in Section VI, *wx* mutants of maize (*158*) and *gl* mutants of rice (*157*) lack the major starch granule-bound starch synthase activity. However, *wx* maize granules do contain a minor granule-bound ADPG–starch synthase (*159*) and two soluble ADPG–starch synthases (*161*).

### 2. Amylose-Extender

Mutant genes, which cause an increase in apparent amylose percentage in starch of pea cotyledons and of maize and barley pollen and endosperm, have been reported (225). High-amylose maize is homozygous for the *ae* gene, and the mature kernels are sometimes reduced in size (Table IV). High-amylose peas are homozygous for the *rugosus* (*r*) gene and have a wrinkled, collapsed phenotype (*226*), while high-amylose barley kernels appear similar to *normal* and are homozygous for the *amylose-1* (*amy-1*) gene (R. F. Eslick, personal communication). Starch and dry weight production are reduced and sugars increased in these high-amylose genotypes compared to nonmutant kernels or seeds. The rate of starch increase is also slightly reduced (*46, 96, 97, 106, 204, 208*). Apparent amylose content increases with increasing maize and barley kernel age and with increasing pea diameter, reaching values of 45–69% (*46, 96, 97, 106*). The *normal* alleles are not completely dominant to the recessive *ae* and *amy-1* alleles, since two doses of the recessive allele (i.e., *Ae ae ae*) result in a 2–8% increase in apparent amylose content compared to the *normal* genotype which lacks the recessive allele (*96, 223, 227, 228*). Extensive variation in apparent amylose concentration occurs compared to the amount observed in *normal* genotypes (see Section III). For example, variation is observed for amylose concentration as a function of the maize inbred crossed with *ae* (*229–233*) with a range of 36.5–64.9% reported (*230*). Minor modifying genes in the various inbreds have



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been proposed as a possible cause of the variation (*229–233*). Such modifying genes have been utilized to produce a series of hybrids which differ in apparent amylose concentration from 50% to 75% (*3*). Differences also have been associated with *ae* alleles arising as independent mutants, with *ae-i1* and *ae-i2* conditioning lower amylose percentages than five other alleles when compared in two isogenic backgrounds (*234*). Amylose percentage also varies 17% among wrinkled seeded pea cultivars (*46, 82*).

Not only does variation occur between *ae* inbred lines and hybrids (i.e., background or modifier effects) and *ae* alleles, but an 8–14% range also exists within an inbred line homozygous for *ae* and grown at a single location in a single year (*230, 233*). This is likely due to a combination of error in amylose determination, segregation of modifier genes which were not yet homozygous (*235*), and the microenvironment of each plant.

Significant differences in *ae* amylose percentage result from both location and year of production with the effect of location considerably greater than that of years (*112, 236*). Later planting dates are associated with higher *ae* amylose percentages; however, poorer agronomic performance negates the value of the increase (*237*). Minor mechanical damage to the plants has little effect on amylose concentration with only a 1–3% reduction caused by extreme leaf defoliation (*238*).

Variation in amylose concentration is also observed between butt, center, and tip zones within individual *ae* ears with the highest percentage in kernels taken from the butt of the ear and the lowest percentage in kernels from the tip zone (*239*). In addition, when the endosperm tissue is divided into tip, middle, and crown portions, the middle portion is highest in amylose percentage within each ear zone (*239*).

The amylose percentages presented above are all based on "blue-value" tests. Yeh and co-workers (*71*) recently employed column chromatography using Sepharose 2B-CL to fractionate the starch polysaccharides from mature *normal, ae*, and 14 other maize endosperm mutants. Column fractions were reacted with iodine, and the absorptions at 560 and 615 nm were determined. Any fraction having a higher absorption at 560 than at 615 nm was classified as amylopectin; and, conversely, fractions with a higher absorption at 615 nm were considered to be amylose. Based on the elution profile, she found considerable carbohydrate intermediate in size (up to fraction 25) between amylopectin and amylose, which absorbed higher at 560 than at 615 nm. These fractions appear similar to the loosely branched amylopectin described for *ae wx* starch (*69, 214*) and suggested for *ae* amylopectin (*240, 241*). Whistler and Doane (*79*) isolated such a polymer from *ae* starch. Low-molecular-weight polymers similar to the short chain amylose described by Banks and Greenwood (*3*) eluted near the end of the profile; these polymers had a higher absorbance at 560 nm than at 615 nm and by definition were not included as amylose. Based on Yeh's calculation, *ae* starch contains 33% amylose (Table VIII) (*71*). If the low-molecular-weight polymers

eluting after amylose are included, the amylose percentage increases to 41%, which is still much lower than amylose percentages based on blue-value measurements (Table VII). Similar low amylose percentages are obtained following gel filtration after debranching by isoamylase (*216*). Because the long external chains of loosely branched polysaccharides complex iodine (*69*), they contribute to the estimate of amylose percentage as measured by the blue-value procedure. Although the amylose percentage based on Yeh's (*71*) and Ikawa and co-workers' (*216*) procedures may not be exact, they probably represent a much closer estimate of the true amylose content of *ae* starch than do blue-value estimates.

Starch granule preparations from *ae* kernels generally contain two distinct geometric forms, spherical and irregular (*26, 36, 97, 225, 242*). Irregular granules vary in shape, but often are elongated and nonbirefringent. Sometimes spherical granules also develop elongated extensions of amorphous, nonbirefringent starch (*39*). The proportion of irregular granules in *ae* starch has been reported to vary from 0% (*26, 71, 97*) to 100% (*243*) and was shown to increase during kernel development (*27, 97*), with increasing apparent amylose content (*97, 225*) and with physiological age of the cells (*36*). The proportion of irregular granules depends on the completeness of starch isolation, on the classification criteria used (*242*), and on the inbred background (*26, 71*). Average *ae* starch granule size increases with kernel development; however, *ae* granules are smaller than *normal* at all developmental stages (*39, 199*). Boyer and co-workers (*36*) reported a two-phase growth pattern consisting of spherical granule initiation and growth followed by a secondary initiation of irregular granules. Sandstedt (*244*) also indicated that *ae* irregular granules are surrounded by spherical granules within an endosperm cell. There is considerable cell to cell variation in the presence and proportion of irregular granules (*36, 244*); but in kernels harvested 36 days post-pollination, the proportion of irregular granules is highest in the more mature endosperm cells (*36*).

Inbred background apparently influences the morphology of the irregular granules produced by *ae*. The elongated amorphous granules noted above occur when the *ae* mutation is incorporated into dent backgrounds (*27, 36*). However, when *ae* is incorporated into the sweet corn inbred 'IA5125' and the *su* mutant deleted, no elongated amorphous granules are found at 16 or 27 days after pollination (*26*) or at maturity (*71*). Secondary granule initiation does occur, and the irregular granules are more blocky in appearance (*26*). Kernels of *ae* have the major developmental gradient and type I minor gradient characteristic of *normal* (*26, 27*).

Starch granules from *ae* kernels have a much higher BEPT than *normal* or the other mutants (Table V). Also, based on 14 genotypes studied, the B-type x-ray diffraction pattern appears to be unique to *ae* and the *ae* containing genotypes (Table V).

In high-amylose barley (*228*) and wrinkled-seeded peas (*46*), average granule size is less than in *norm* of development. High *normal* (*228*). High-ar system of fissures, ma *46, 124*).

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size is less than in *normal* with high-amylose starch granules smaller at all stages of development. High-amylose barley starch granules are more irregular than *normal* (*228*). High-amylose pea starch granules often develop a very irregular system of fissures, making them superficially resemble compound granules (*1, 46, 124*).

Based on the accumulation of loosely branched amylopectin in *ae* (*240, 241*) and *ae wx* (*69, 214*) genotypes, Boyer and co-workers (*96*) suggested that the *Ae* allele affects the degree of branching of amylopectin by controlling the quantity of an effector, at the site of starch synthesis, which stabilizes a starch synthase–branching enzyme complex. According to this suggestion, the enzyme complex is needed for production of *normal* amylopectin. The *ae* allele-gene product may block effector accumulation resulting in increased free branching enzyme and free starch synthase. This is in agreement with the observations of Schieffer and co-workers (*170*) who showed with zymograms that starch synthases of *ae* have increased activity in the free synthase bands and a decreased activity in the starch synthase–branching enzyme complex bands. Boyer and co-workers (*96*) suggested that citrate may function as the effector compound which stabilizes the enzyme complex *in situ*. However, malate rather than citrate was shown to accumulate in *normal* maize amyloplasts, and Liu and Shannon (*131, 150*) suggested that malate may be the effector compound functioning *in situ*. Although the *ae* mutant is clearly influencing the efficiency of branching, Boyer and co-workers (*96*) point out that their hypothesis for *ae* action is only a suggestion and that the assignment of a positive gene product to *ae* awaits direct evidence.

Recently, Boyer and Priess (*169*) reported the presence of three forms of branching enzyme in extracts from *normal* maize endosperm. One component, fraction IIb, coelutes with the citrate-stimulated, "unprimed" starch synthase. When the branching enzymes from *ae* kernels were similarly separated, the total activity was only 20% of *normal*, and there was a complete absence of branching enzyme fraction IIb (*171*). Based on these results, Nelson (*5*) concluded that the missing branching enzyme activity in *ae* could explain the effects of *ae* on the polysaccharides formed. More recently, Boyer and co-workers (*291*) showed that, with increasing doses of the recessive alleles, *ae* in maize and $r_a$ in peas, there was an increase in the linearity of amylopectin produced. In maize, this *ae* effect on amylopectin was apparently due to the deficiency of branching enzyme IIb (*171*). Hedman and Boyer (*292*) reported a near-linear relationship between increasing dosage of the dominate *Ae* allele and branching enzyme IIb activity and suggested that *ae* is the structural gene coding for branching enzyme IIb. The high-amylose wrinkled pea, Progress #9, has greatly reduced levels of branching enzyme (*289*). Thus, the presence of modified amylopectin in the "high-amylose" mutants in both species owes to the reduced activity of branching enzyme.

### 3. Sugary

The standard sweet corn of commerce is homozygous recessive for *su*. The main effect associated with *su* mutants in maize and sorghum is the synthesis and accumulation of phytoglycogen to 25% or more of the kernel dry weight (Table VI) (*13, 205, 245, 246*).

Phytoglycogen consists of α-D-glucosyl units linked by (1→4) and (1→6) bonds. Its structure is similar to that of amylopectin, except that phytoglycogen is more highly branched and is extracted as the major component of the water-soluble polysaccharide (WSP) fraction in sweet corn (*13, 247, 248*). Mature *su* sorghum and maize (Table IV) kernels are wrinkled and have reduced amounts of dry matter (*205, 208, 212, 249*). Their sugar content is higher and their starch content much lower than in *normal* maize (*204, 205, 208, 250–252*) or sorghum (*246, 249, 253*). Starch concentration in *su* maize expressed as a percentage of dry weight increases until 15–20 days post-pollination, and then remains constant (*41, 204, 251, 254*). Total polysaccharide concentration, however, increases through 30–40 days post-pollination due to increases in phytoglycogen concentration, with total carbohydrate percentage approaching that in *normal* kernels (*41, 204, 251, 254*). At maturity, the total carbohydrate percentage is equal to (*252, 254*) or less than that in *normal* kernels (*205, 246*), depending on the genetic background. However, absolute amounts are reduced, reflecting the reduced dry matter in *su* kernels. In general, maize kernels from dent lines homozygous for *su* contain more sugar and less phytoglycogen and starch than kernels of a sweet corn line (*204, 205*).

The amylose percentage of starch, as measured by iodine binding, from *su* kernels averages somewhat higher than the percentage from *normal* kernels (Table VII), and the amylose percentage has been reported to increase with advancing kernel age (*41, 255*). Although the data in Table VII represent data from several studies over several years, other investigators have reported widely different amylose percentages in *su* starch (*71, 206, 208, 251, 256–258*). These have varied from 0% amylose (*251*) to 65% amylose (*71*). The 65% amylose, reported by Yeh and co-workers (*71*) (Table VIII), was based on calculations from a Sepharose separation of the starch polysaccharides. Similarly, the amylose percentage of starch from *su* sorghum kernels varied from near *normal* (*86*) to somewhat higher than *normal* (*253*). The widely differing amylose percentages probably relate to kernel age and methods of starch isolation and measurement. Possible reasons for these discrepancies will be discussed in more detail after considering the morphological changes occurring in the developing *su* kernel.

The morphology and development of *su* maize plastids and kernels is well established (*1, 9, 23, 26, 27, 128*). Immediately prior to initiation of starch synthesis in an endosperm cell, the proplastids collect around the nucleus as in

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*normal* (*26, 27, 128*). From one to several small starch granules then form in each amyloplast (*128*). During development, granules enlarge only slightly (*41, 199, 255*), reaching an average diameter of 3.6 μm at maturity (*41*). However, within the more mature cells of the central crown region, the initially formed starch granules are degraded and are replaced with phytoglycogen (*26, 27, 128*). Thus, within developing kernels, plastid types range from amyloplasts with compound starch granules, to amyloplasts containing phytoglycogen and a few small starch granules, to amyloplasts containing phytoglycogen plus many very small starch granules and/or granule fragments, and to plastids containing only phytoglycogen (*26, 27, 128*). The cells with the different plastid types are located in specific regions of the endosperm and apparently are related to the physiological age of the cells with phytoglycogen plastids being in the most mature cells (*23, 26, 27*). The *su* kernels go through the major and minor developmental sequence characteristic of *normal*, except that, as the cells mature, they fill with phytoglycogen rather than starch (*26, 27*). Later in kernel development, phytoglycogen plastids in some cells appear to rupture (*26, 27, 128*). The released material, thought to be phytoglycogen, was described as a dense-staining "rosette" material (*128*) similar in appearance to animal glycogen (*259*). Thus, phytoglycogen appears to accumulate in both plastids and the cytoplasm, with that in the cytoplasm possibly arising from ruptured plastids.

Owing to the small size of *su* starch granules (Table V) and their partially degraded reminants, difficulties are encountered in isolating a starch sample which is representative of that in the total population of cells found in the endosperm. With procedures involving starch-tabling, up to 90% of the starch can be lost (*260*), and similar losses of the smaller particles would be expected with isolation procedures based upon low-speed centrifugation or gravity sedimentation. Particles staining both red and blue with iodine have been observed *in situ* and in isolated granules (*26, 260, 261*). Thus, the granules differ from each other, and loss of small granules and granule particles probably results in isolated granule preparations which are not representative of the total granule population. Differences in isolation procedures used by different investigators may explain some of the discrepancy in amylose percentages reported for *su* starch. The amylose percentage in the starch also is affected by the completeness of phytoglycogen removal. Polysaccharide particles smaller than starch granules have been observed in *su* kernels (*26*) and also have been isolated from immature kernels (*258, 293*). These intermediate particles, composed of phytoglycogen and amylose (*258*), cause a further difficulty in accurately estimating the amylose percentage in starch and in the characterization of phytoglycogen. If these particles are considered to be starch granules, the amylose content will be underestimated. If they are collected with the phytoglycogen fraction, amylose will be found; a phenomena which has been reported (*262, 293*). Thus, kernels homozygous for the *su* gene cannot be considered to contain only phytoglycogen

and starch granules, but also must be considered to have a range of particles with intermediate composition resulting from the partial conversion of starch granules into phytoglycogen.

Several investigators (*13, 171, 263–265*) have reported the presence of a branching enzyme (phytoglycogen branching enzyme) in *su* kernels, in addition to Q-enzyme, which is capable of forming a phytoglycogen-like polysaccharide from amylose *in vitro*. Black and co-workers (*13*) observed the presence of phytoglycogen branching enzyme in all maize genotypes containing phytoglycogen and in two mutants (*du* and *wx*) which do not accumulate phytoglycogen. Of the three branching enzymes present in maize kernels, Boyer and co-workers (*294*) suggest that branching enzyme I plays a major role in phytoglycogen formation. However, there is a specific interaction between branching enzyme I and starch granules from *su* kernels. For example, treatment of *su* starch granules with this enzyme causes the formation and release of phytoglycogen-like glucans, but no soluble glucan was released from enzyme-treated non-mutant starch granules (*294*). Black and co-workers (*13*) concluded that the gene *su* is not the controlling factor, either in the formation of phytoglycogen or of the phytoglycogen branching enzyme. Nelson (*5*) agrees that the *su* locus is not the structural gene for the phytoglycogen branching enzyme.

A complex multiple allelic series exists at the *su* locus in maize, and four phenotypic categories have been established for mature kernels based on examination of 12 independently occurring mutations (*266*). For most alleles, mature kernels resemble the reference allele, *su-Ref*, discussed in the preceding paragraphs (Table IV) (*266*). Kernels of three alleles, including *su-am* (*amylaceous*), are near-normal in appearance and are best observed as double mutants with *du* or *su2* (*261, 266–268*). Kernels of *su-st* (*starchy*) vary from near-normal to slightly wrinkled with *su-st* recessive to *su-Ref* in some backgrounds (*266, 269*). The fourth class, represented by *su-Bn2* (*Brawn-2*), has a kernel phenotype intermediate between *su-Ref* and *su-am* (*266*). This phenotype complexity is

**Table IX**

*Dry Weight and Carbohydrate Composition of Kernels Sampled 20 Days Postpollination for Alleles at the Sugary Locus Converted to the W64A Dent Inbred Background*[a]

| *Sugary allele* | *Ears sampled, no.* | *Kernel weight, mg* | *Glucose*[b] | *Fructose*[b] | *Sucrose*[b] | *WSP*[b] | *Starch*[b] |
|---|---|---|---|---|---|---|---|
| *su-Ref* | 3 | 27 | 45 | 39 | 245 | 130 | 77 |
| *su-Bn2* | 8 | 33 | 41 | 36 | 177 | 55 | 241 |
| *su-st* | 7 | 27 | 60 | 54 | 124 | 122 | 191 |
| *su-am* | 7 | 36 | 60 | 52 | 78 | 4 | 356 |

[a] D. L. Garwood and S. F. Vanderslice (*295*).
[b] Milligrams per gram of dry weight.

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reflected in the carbohydrate composition conditioned by these alleles with composition ranging from that of *normal* for *su-am* to that exhibited by *su-Ref* (Table IX). Based on the existence of multiple alleles which condition unique carbohydrate compositions, Garwood and Vanderslice (*295*) hypothesized that the *su* locus is a regulator locus.

An independent recessive modifier of the *su* locus, named *sugary enhancer* (*se*), has been described in the sweet corn line 'IL677a' (*270, 271*). The resulting *su se* genotype accumulates high sugar levels similar to *sh2* and also high levels of phytoglycogen similar to *su-Ref* (*254, 270, 271*).

### 4. Sugary-2

Kernels of the maize endosperm mutant *su2* have a slightly tarnished phenotype (Table IV) and are similar to *normal* in soluble sugar, WSP (Table VI), and starch concentrations during development (*204, 208*) and at maturity (*205*). Kernel dry weight is often (*204, 208, 212*), but not always (*205*), reduced. Starch granule size (Table V) (*204, 256*) and rate of size increase during development (*199*) are similar to *normal;* however *su2* granules have extensive internal fractures (*260*). Starch from *su2* endosperms is 10–15% higher in apparent amylose content than is *normal* starch (Table VII), with the *normal* (*Su2*) allele completely dominant to *su2* (*222–224*). As with other genotypes, year of production (*224*), *su2* allele examined (*223*), the background into which *su2* is incorporated (*230*), and different ears within a *su2* inbred (*230*) affect apparent amylose percentage. Although *su2* starch composition is altered, purified *su2* amylose and amylopectin have properties similar to those of *normal* amylose and amylopectin (*256*). Singh (*253*) has described a sorghum mutant similar to *su2*, which also has nonmutant levels of reducing sugars, WSP, and starch, but is higher in sucrose and amylose percentage.

Brown and co-workers (*199*) reported that starch granules from 18- and 24-day-old *su2* kernels are weakly birefringent and have an A-type x-ray pattern (Table V), in contrast to the B-type pattern reported for starch granules from mature *su2* kernels (*256, 272*). The BEPT of *su2* granules is lower than that of *normal* granules (Table V) (*206, 273*). Based on these granule properties, the *su2* gene has been suggested to cause a reduction in the molecular association between the starch molecules of the granule (*199*); however, no genetic lesion has been established for *su2*.

### 5. Dull

The *du* mature kernel phenotype varies with background, ranging from full size to semi-collapsed (Table IV). The presence of the "normal appearing" form is best detected in combination with *su-am* (*261, 267, 274*). The more extreme expression may be associated with the presence of a dominant *dull-modifier* gene

(*274*). Mature kernel dry weight of *du* also varies, with some weights similar to those of *normal* (*204*, *213*) and others significantly less (*212*). The sugar concentration is slightly higher and the starch concentration lower than *normal* in both immature (*204*, *208*) and mature (*205*) kernels.

The amylose percentage of *du* starch in a dent background is 5–10% higher than the percentage in *normal* starch (Table VII). Yeh and co-workers (*71*) reported 55% amylose in starch from mature *du* kernels in a sweet corn background (Table VIII). Differences in these values may be due to the Sepharose separation procedure used by Yeh and co-workers (*71*) or to a background effect. The *normal* (*Du*) allele is completely dominant to *du* for amylose percentage (*221*, *223*, *224*). The amylose percentage is affected by the *du* allele (*223*), by the background (*230*), and by the year of production (*224*). Although the amylose percentage is higher than in *normal*, the polysaccharide components have similar properties (Table VIII) (*256*).

Most *du* granules are similar in shape, size, birefringence, and iodine staining to *normal* granules (*26*, *256*, *260*); however, some irregularly shaped granules and spherical granules, which have little or no birefringence, have been reported (*26*, *260*). Average *du* starch granule size is smaller than *normal* granule size (Table V) (*204*). Cell to cell variation in granule size and morphology has been reported (*260*). BEPT and x-ray diffraction patterns are similar for *du* and *normal* (Table V) (*206*).

Saussy (*26*) studied *du* kernel and plastid development in a sweet corn background. The *du* kernels have a major developmental gradient similar to *normal* except for the presence of slender, thin-walled cells near the developing embryo which appear partially compressed. Although *du* kernels in a dent background do not accumulate phytoglycogen (*13*), those in a sweet corn background do have cells in the central endosperm with plastids containing phytoglycogen and one or two small starch granules (*26*). Secondary initiation of granules has been observed in some cells (*26*). Kernels of *du* have a type II minor developmental gradient from the outside toward the interior (*26*) in which there is typical starch granule initiation and enlargement for a few cell layers, followed by an abrupt reduction in number and size of starch granules. The reduction in starch is accompanied by an increase in phytoglycogen containing plastids. All multiple mutants homozygous for *du*, but none of the others examined, had the type II minor gradient, and Saussy (*26*) suggested that this property was a specific effect of the *du* gene.

Phytoglycogen branching enzyme has been found in *du*; however, no phytoglycogen was isolated by Black and co-workers (*13*). Priess and Boyer (*275*) reported that the *du* mutation lowered the starch synthase II activity and also lowered branching enzyme IIa activity. Because the activities of two enzymes are diminished by *du*, it is possible that *du* may be a regulatory type gene, but a specific genetic lesion has not been associated with it.

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### 6. Amylose-Extender Waxy

The *ae wx* mature kernel phenotype is reduced in size compared to *normal* (Table IV). Similarly, immature and mature kernel dry weights and starch contents are reduced almost 50% (*96, 204, 205*); however, sugar contents are increased (*204, 205, 276, 277*). Only small amounts of material are recovered in the WSP fraction (Table VI) (*276, 277*).

Apparent amylose percentages of 15–26% have been determined for *ae wx* using the blue-value procedure (Table VII), and it was originally thought that *ae wx* is the only genotype producing a significant quantity of amylose when *wx* is homozygous (*208*). However, using potentiometric titration, only 1% amylose was observed, indicating little linear material was present (*223*). This finding was confirmed by chromatographic separations on Sepharose and fine structure analyses which showed that *ae wx* starch consisted solely of loosely branched amylopectin with long external chains (*69, 70, 214*). A similar loosely branched polysaccharide of lower molecular weight also is found in *ae wx* starch in a sweet corn background (Table VIII). Thus, in this double mutant, the *wx* gene is blocking all accumulation of linear polymer, while the *ae* gene is interfering with typical branching. The enzymic reactions discussed under the respective single mutants apparently are both functioning independently in the double mutant.

Increasing doses of *ae* and *wx* effect kernel phenotype (*278*) and amylose content (*96, 223*). Two or 3 doses of the *wx* allele significantly decrease apparent amylose, indicating tighter branching, while 2 or 3 doses of the *ae* allele significantly increase apparent amylose content, indicating looser branching, regardless of the gene dosage at the other locus (*96, 223*). Apparent amylose content of *ae wx* starch decreases with increasing kernel age (*39, 96*), indicating tighter branching. Different *ae* alleles combined with *wx* may also affect the degree of branching, since pollen from different *ae wx* combinations differs in iodine staining (*279*).

Kernels of *ae wx* have the major and minor (type I) developmental gradients characteristic of *normal* (*26, 27*). Starch granules are smaller than *normal* (Table V) and increase somewhat in size with increasing kernel age (*39, 199*). Considerable differences relative to starch granule and plastid development have been observed between dent and sweet corn backgrounds (*26, 27*). In a dent background, no secondary granule initiation, characteristic of *ae*, is observed, but most granules within a cell seem to develop extensions simultaneously (*27*). These granules remain highly birefringent (*27*). In contrast, Brown and coworkers (*199*) reported that the spherical *ae wx* granules have a polarization cross, while the irregular granules only have birefringence on the outer periphery. No phytoglycogen containing amyloplasts are observed in *ae wx* kernels in a dent background (*27*). In a sweet corn background, secondary granule initiation is observed, and many amyloplasts contain a starch granule surrounded by a

noncrystalline polysaccharide (*26*). Staining properties of this polysaccharide are similar to those of phytoglycogen. "Phytoglycogen" containing plastids of *ae wx* persist to maturity and, unlike the phytoglycogen plastids in *su* kernels, many of the purified starch granules are still surrounded with the "phytoglycogen-like" polysaccharide (*71*). The nature of this polysaccharide is unknown, but it may be similar to that observed in the triple mutant *ae du wx* to be described later.

### 7. Amylose-Extender Sugary

Mature kernels of *ae su* are not as full as *ae*, but are fuller than *su* (Table IV); and their phenotype varies with genetic background (Table IV) (*280*). Kernel dry weight and starch concentration are reduced relative to *normal* (*204*, *205*). Sugar concentrations are slightly higher than those of *normal* in immature (*204*), but not in mature (*205*), kernels. Minimal WSP levels have been reported in *ae su* and were similar to those in *normal* (Table VI); however, in subsequent studies, significant amounts of phytoglycogen were found (*26*, *281*). Specifically, in a dent background *ae su* endosperm contains 11% as much phytoglycogen as *su* endosperm at 20 days post-pollination. Increasing doses of *ae* in a homozygous *su* genotype results in reduced levels of phytoglycogen (*281*). Kernels of *ae su* in a sweet corn background have a large area of cells containing plastids with starch granules surrounded by a non-crystalline "phytoglycogen-like" polysaccharide (*26*). Only a few such plastids were observed in a dent background (*27*). Thus, background is important in the degree of *ae* epistasis relative to the accumulation of "phytoglycogen-like" polymers.

Starch from *ae su* kernels in a dent background consists of 51–60% amylose as determined by the blue-value procedure (Table VII), with the amylose percentage increasing with increasing kernel age (*39*). Yeh and co-workers (*71*), in contrast, reported that *ae su* reduced amylose concentration from 65% for *su* to 28% for *ae su*, based on Sepharose separation of starch polysaccharides isolated from kernels in a sweet corn background. Three fractions were obtained (Table VIII). The first two were loosely branched similar to the amylopectin fractions in *ae*. Amylose from the third peak fraction was similar in iodine staining to that from *normal;* however, some short-chain-length amylose was present as found in *ae*. The second fraction from the Sepharose column eluted in the same position as phytoglycogen and may have been the noncrystalline "phytoglycogen-like" polysaccharide shown to be present with some of the "purified" starch granules (*71*). However, the iodine complex absorption maximum of this lower-molecular-weight branched component was the same as that of the first component and similar to the branched components of *ae wx* (Table VIII). Neither branched component from *ae su*, when complexed with iodine, had an absorption max-

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imum even close to that for *su* phytoglycogen (Table VIII). A similar lower-molecular-weight loosely branched component comprising 7.5% of *ae su* starch has been isolated (*79*).

Kernels of *ae su* have the major and minor (type I) developmental gradients characteristic of *normal* (*26, 27*). Starch granules are smaller than *normal* (Table V) and increase in size with increasing kernel age (*39, 199*). Secondary granule initiation occurs in *ae su* kernels similar to that in *ae* (*26, 27*). Within some cells in a dent background, granules are transformed during development into an amorphous nonbirefringent form (*27, 260*). Badenhuizen (*9*) reported that spherical granules from young kernels have an A-type x-ray pattern; but with development, irregular granules with a B-type x-ray pattern are found. Starch granules and plastid development in *ae su* kernels in a sweet corn background vary considerably from cell to cell (*26*). Some cells contain irregular granules; others contain granules surrounded by "phytoglycogen-like" polysaccharide, and others have plastids with granules in various stages of fragmentation.

The effects of both genes can be seen in the double mutant. Phytoglycogen, as found in *su*, is produced; however, amounts are reduced in *ae su*, although to a lesser degree in a sweet corn background. The *ae* gene reduces branching, which is reflected in the two loosely branched starch fractions obtained by Sepharose chromatography (Table VIII). Furthermore, *ae* probably interferes with phytoglycogen branching, for *ae su* phytoglycogen is degraded more by β-amylase than is the *su* phytoglycogen (*281*). In *su*, the initially formed starch granules are broken down and are thought to be utilized in the production of phytoglycogen. In *ae su*, the *su* gene may be responsible for causing the partial breakdown of the initially formed starch, but *ae* interferes with branching and an amorphous irregular granule is formed in a dent corn background along with a small amount of phytoglycogen (*27*). In the sweet corn background, more "phytoglycogen-like" polysaccharides are formed, apparently because of modifier genes (*26*).

### 8. Amylose-Extender Sugary-2

The mature kernel phenotype of *ae su2* is similar to that of *ae* (Table IV). Dry weight per kernel is similar to that of *su2* and *normal*, while starch concentration is less than that of *su2* and similar to that of *ae* (*204, 205*). Sugar concentrations in immature (*204*) and mature (*205*) kernels are higher than those in either *ae* or *su2*. Amylose percentage, based on blue-value determinations, is similar to that in *ae* (Table VII). Amylose percentage varies between *ae su2* ears (*230*), although *su2* or *ae* alleles have little effect on *ae su2* amylose percentage (*223*). No dosage effects are observed (*223*). Starch granule sizes and x-ray diffraction patterns are similar to those in *ae*, and the BEPT approaches that of *ae* (Table V) (*206*).

### 9. Amylose-Extender Dull

The phenotype of mature *ae du* kernels differs from that of both *du* and *ae* (Table IV). Compared to *normal*, dry weight and starch concentration are reduced, while sugar concentration is higher in immature (*204*) and mature (*205*) kernels. The amylose percentage of *ae du*, based on blue-value measurements of starch from kernels in a dent background, is similar to that in *ae* (Table VII). With *ae* homozygous, the apparent amylose percentage decreases with increasing doses of *du* (*223*). The 47% amylose determined by the Sepharose separation of starch from *ae du* kernels in a sweet corn background is intermediate between the amount in *ae* and *du* (Table VIII). The maximum absorption of the iodine–amylopectin complex of *ae du* is similar to that of *ae*, while the amylose component is closer to that of *du* and *normal* (Table VIII). Thus, also in *ae du*, the *ae* gene appears to be interfering with the typical branching of amylopectin resulting in the production of more loosely branched polymers.

Although low levels of WSP have been reported in *ae du* kernels (Table VI), Black and co-workers (*13*) concluded that no phytoglycogen accumulates in *ae du* kernels in a dent background. In contrast, kernels of *ae du* in a sweet corn background produce numerous plastids with one or two starch granules surrounded by a thick layer of noncrystalline "phytoglycogen-like" polysaccharide (*26*).

Kernels of *ae du* in a sweet corn background are slightly delayed in development, but have the *normal* major gradient of kernel development (*26*). The type II minor gradient characteristic of *du* is observed in *ae du* (*26*). Saussy (*26*) also reported that secondary starch granule initiation occurs and that granules assume a blocky, elongated irregular shape later in development.

In a dent background, the greatest increase in granule size occurs between 12 and 18 days post-pollination (*199*). Granule size is similar to that of *ae* and *du* granules, but less than that of *normal* granules (Table V). The *ae du* starch granules have a B-type x-ray defraction pattern similar to that of *ae* (Table V). In contrast, the *ae du* BEPT is similar to that of *du* (Table V) (*206*, *273*). In *ae du*, the *ae* and *du* genes appear to be functioning independently with *ae* interfering with typical branching, and *du* causing the expression of the type II minor gradient. In *ae du*, branching enzyme fractions IIa and IIb and starch synthase fraction II are considerably reduced (*275*). Thus, the double mutant expresses the enzyme reductions of both individual mutants.

### 10. Dull Sugary

The mature kernel phenotype of *du su* is similar to that of *su*, although *du su* kernels are often more wrinkled (Table IV). This genotype has been extensively studied to evaluate its potential for improving sweet corn quality (*282*, *283*).

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Compared to *normal*, *du su* kernels have reduced dry weight and starch concentration and increased sugar and WSP concentrations (*204*, *205*). Sugar (*204*, *205*, *261*, *270*, *282*, *283*) and WSP (Table VI) (*261*, *282*) levels are similar to those in *su*, although starch (*205*, *261*, *282*) concentration is lower. Thus, *su* is epistatic to *du* relative to phytoglycogen accumulation.

Widely varying amylose percentages have been reported for *du su* starch samples in a dent background when measured by the blue-value procedure (Table VII). In four other reports, *du su* amylose content ranged from 51% to 66% (*222*, *224*, *256*, *261*). Yeh and co-workers (*71*) (Table VIII) reported 70% amylose using Sepharose column chromatography. Dvonch and co-workers (*256*) stated that *du su* amylopectin is intermediate in branching between glycogen and *normal* amylopectin. However, based on the absorption maximum of the iodine complex and the extinction coefficient, the high-molecular-weight branched fraction from *du su* in a sweet corn background appears to be loosely branched with long external chains (Table VIII). The *du su* amylose fraction is similar to that of *du* and *su* alone. No dosage effects have been observed on amylose percentage (*224*).

The overall kernel and plastid development pattern in *du su* is similar to that in *su* except that *du* causes the type II minor gradient (*26*). Compound or semicompound granules are initially formed, followed by slight enlargement, fragmentation, and accumulation of phytoglycogen. At later stages of development in some cells, the phytoglycogen plastid membrane ruptures as in *su*, and the phytoglycogen mixes with the cytosol. Saussy (*26*) also reported a lack of secondary granule initiation. No increase in the average size of *du su* starch granules is observed between 12 and 24 days post-pollination (*199*). The *du su* granules are similar in size to those of *su* (Table V). This lack of size increase is probably due to the observed granule fragmentation (*26*). Granules isolated from mature kernels show weak or no birefrigence (*71*), and those from 24-day-old kernels have a weak A-type x-ray diffraction pattern (*199*).

### 11. Dull Sugary-2

The mature kernel phenotype of *du su2* differs from both *du* and *su2* (Table IV). The sugar and WSP (Table VI) concentrations in immature (*204*) and mature (*205*) *du su2* kernels are similar to those in *du* and *su2*, except that, in immature *du su2* kernels, the sugar concentration is higher than that in *su2*. Starch concentration is lower than that in either *du* or *su2* (*204*, *205*); and the amylose percentage, as measured by the blue-value test (Table VII) or by potentiometic titration (*256*), is higher than that in either *su2* or *du*. No dosage effects on amylose percentage have been observed (*224*). Isolated *du su2* amylose and amylopectin have properties similar to those of *normal* (*256*); however, Whistler and Doane (*79*) isolated 8.7% of *du su2* starch in a loosely branched amylopectin

fraction. Average size of *du su2* starch granules is similar to that of the single mutants (Table V). The BEPT of *du su2* starch granules is similar to that of *su2* granules (Table V) (*273*) and *du su2* granules have an A-type x-ray diffraction pattern (Table V).

### 12. Dull Waxy

The mature *du wx* kernel phenotype differs from that of either *du* or *wx* (Table IV). Dry weights of mature kernels are similar to those for *du* and *wx* and slightly less than those of *normal* (*205*). Sugar concentrations are higher, and starch concentration is lower than in either *normal*, *du*, or *wx* in immature (*204*) and mature (*205*) kernels.

Starch in the double mutant *du wx* is essentially 100% amylopectin; thus, the *wx* mutant is epistatic to *du*. The absorption maximum and extinction coefficient of the *du wx* branched polysaccharide–iodine complex are the same as for *wx* (Table VIII). When the *wx-a* allele is combined with *du*, *du wx-a* starch contains 9% amylose, reflecting the increased amylose conditioned by the *wx-a* allele alone (*221*).

Neither *du* nor *wx* in a dent background accumulates phytoglycogen (Table VI), but they both contain phytoglycogen branching enzyme (*13*). However, when combined in the double mutant *du wx*, immature kernels contain up to 11% phytoglycogen (Table VI). Although not quantatively determined, Saussy (*26*) reported numerous phytoglycogen-containing plastids in endosperm cells of *du wx* in a sweet corn background.

Starch granule size of *du wx* at 18 and 24 days postpollination is intermediate between *du* and *wx* (Table V). The mean BEPT and A-type x-ray diffraction pattern of *du wx* starch are the same as for *normal* and the component single mutants (Table V) (*206*).

Kernels of *du wx* in a sweet corn background have the major developmental gradient typical of *normal* and a type II minor gradient characteristic of du (*26*). Secondary granule initiation is observed in many cells. Granule shapes vary from spherical to irregular-blocky. Plastids containing starch granules surrounded by phytoglycogen are generally located in the more mature cells of the central endosperm region (*26*).

### 13. Sugary Waxy

The *su wx* mature kernel phenotype is similar to that of *su* (Table IV). Immature (*204*, *250*) and mature (*205*) kernel carbohydrate composition is similar to that in *su*, except that *su wx* starch is composed of 100% amylopectin (Table VII). The starch component in *su wx* has properties similar to those of both *wx* starch and the amylopectin component of *su* starch (Table VIII) (*256*). With *su* homozygous, increasing doses of *wx* reduce amylose concentration (*222*). The



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WSP content (Table VI), β-amylolysis limits, and chain lengths of *su wx* phytoglycogen are the same as those from *su* (*13*). Immature kernels contain phytoglycogen branching enzyme (*13*). Starch granules isolated from *su wx* are small, aggregated, and compound (similar to *su* granules), and phytoglycogen is completely removed from the starch granules during isolation (*71*). The granules are strongly birefringent (similar to those from *wx*) (*71*). Thus, *wx* is epistatic to *su* relative to the absence of amylose in the starch, and *su* is epistatic to *wx* relative to soluble sugar and phytoglycogen concentrations, kernel phenotype, and starch granule size.

### 14. Sugary-2 Waxy

The mature kernel phenotype for *su2 wx* is similar to that for *wx* (Table IV). Mature (*205*) and immature (*204*) kernel dry weight and carbohydrate composition are similar to those of the single mutants. The *wx* mutant is epistatic to *su2*, resulting in starch with approximately 100% amylopectin (Table VII).

### 15. Sugary Sugary-2

The *su su2* mature kernel phenotype is similar to that for *su* (Table IV). This genotype has been extensively evaluated for its sweet corn improvement potential (*282*, *283*). Immature (*204*, *282*) and mature (*205*) kernel carbohydrate composition approaches that of *su* kernels; however, starch accumulation is reduced (*204*, *282*). The apparent amylose percentage is similar to that of *su2* starch (Table VII). Amylose concentration increases with increasing doses of *su2* when *su* is homozygous and with increasing doses of *su*, when *su2* is homozygous (*222*). Starch granule size (Table V) (*256*) and BEPT (Table V) (*206*) are similar to *su*. Thus, *su2* is epistatic to *su* for apparent amylose concentration, while *su* is epistatic to *su2* for starch granule size, carbohydrate composition, and mature kernel phenotype.

### 16. Amylose-Extender Dull Sugary

The mature kernel phenotype for the triple mutant *ae du su* is similar to that for *su* (Table IV). Sugar concentrations of mature (*205*) and immature (*204*) *ae du su* kernels are higher than those of either of the single mutants or the double mutants *ae du* or *ae su*, while starch concentration is similar to that in *su* and the two double mutants. The amylose percentage is near *normal* when measured by either the blue-value test (Table VII) or the Sepharose separation technique (Table VIII). However, in contrast with *normal*, a major proportion of the branched polysaccharide is smaller than typical amylopectin (as is that of *ae su*), and it elutes from a Sepharose column at an intermediate position (Table VIII). The absorption maximum and extinction coefficient of the branched polysac-

charide–iodine complexes are similar to those for *ae* and *ae su* and are characteristic of loosely branched polymers. The absorption maximum of the amylose–iodine complex is similar to that for *normal*, *du*, or *su*; but the extinction coefficient is lower than for either (Table VIII). No short chain amylose has been found in *ae du su* (*71*).

Phytoglycogen accumulates in *su* and *du su* kernels, but not in *ae* or *du* (Table VI). In the double mutant *ae su*, *ae* is epistatic to *su*, but the addition of *du* allows a somewhat larger amount of phytoglycogen to accumulate (Table VI). Phytoglycogen branching enzyme has been reported in *su* and *du*, but not in *ae* or *ae su* (*13*). Apparently, the branching enzyme activity resulting from the addition of *du* to *ae su* is sufficient to partially overcome the inhibitory effect of *ae* on phytoglycogen accumulation.

Endosperms of *ae du su* in a sweet corn background have the *normal* major developmental gradient and a type II minor gradient characteristic of *du* (*26*). Secondary starch granule initiation has been observed. Starch granules from *ae du su* are similar to those from *du su* and are weakly birefringent (*26*, *71*). Various starch granule shapes from simple spherical to irregular are observed in granules from immature (*26*) and mature kernels (*71*). Starch granule fragmentation and disappearance concomitant with increased phytoglycogen in plastids, characteristic of *su*, also are common in *ae du su* (*26*). Thus, *su* is epistatic to *ae du* relative to plastid type.

### 17. Amylose-Extender Dull Sugary-2

The mature kernel phenotype of *ae du su2* differs from each of the component single or double mutants (Table IV). The sugar and starch concentrations of mature (*205*) and immature (*204*) kernels of *ae du su2* are similar to those of *ae su2* kernels. Sugar concentrations are higher than in the single mutants or other double mutants in this combination, while starch concentration is lower (*204*, *205*). Quantity of WSP is higher in mature and immature kernels of *ae du su2* than in *normal* or any of the single and double mutants in this combination (Table VI). However, Black and co-workers (*13*) did not detect phytoglycogen in *ae du su2*, and the nature of the WSP has not been determined. Apparent amylose percentage of *ae du su2* starch is similar to that in *du* and *su2*, but is lower than that in *ae* starch (Table VII).

### 18. Amylose-Extender Dull Waxy

The mature kernel phenotype of the triple mutant *ae du wx* differs from any of the single mutants (Table IV). Starch concentration is low compared with the component single and double mutants, while sugar concentrations are severalfold higher (*204*, *205*). WSP concentration in *ae du wx* kernels in a dent background is lower than in *du wx*, but is similar to the quantity in the single and other double

mutants (Table VI). Little if any of this WSP is phytoglycogen (*13*). In contrast, amyloplasts from *ae du wx* in a sweet corn background frequently contain one or two starch granules surrounded by a noncrystalline polysaccharide (*26, 71, 131*). The structure of the noncrystalline polysaccharide has not been determined, but the iodine-staining property appears similar to that of phytoglycogen *in situ*. However, in contrast with phytoglycogen in *su* kernels, it is not readily removed from the granules during aqueous granule isolation (*71, 131*). Extraction of the isolated granules with 10% ethanol removes some polysaccharide (*71*). This extracted material is largely composed of branched polysaccharides of intermediate size having the same polysaccharide–iodine complex absorption maximum as the 10% ethanol residual granules, with this maximum higher than that of *su* phytoglycogen (*71*).

These genes have been incorporated into sweet corn inbreds, and a new type of vegetable corn has been introduced which is intermediate in sweetness between standard sweet corn (*su*) and sweet corns based on the *sh2* mutation (*284*). This hybrid, 'Pennfresh ADX,' has the advantage of extra sweetness at harvest and sugar retention for an extended time in storage (*277*).

Starch from *ae du wx* kernels is composed entirely of branched polysaccharides which are largely of intermediate size between amylopectin and amylose (Table VIII). The absorption maximum and extinction coefficients of the polysaccharide–iodine complexes are similar to those of amylopectin from *wx* and *du* rather than those of the loosely branched polysaccharides in *ae*, the *ae* double, and other *ae* containing triple mutants (Table VIII). Both *du* and *wx* kernels contain phytoglycogen branching enzyme (*13*). In combination, they apparently overcome the effect of *ae*, resulting in the production of polysaccharides, both granular and nongranular, which are more highly branched than those of *ae* amylopectin (Table VIII).

Endosperm of *ae du wx* in a sweet corn background has a major developmental gradient typical of *normal* and a type II minor gradient characteristic of *du* (*26*). Saussy (*26*) reports that starch granule and plastid development in *ae du wx* is similar to that observed in *du wx*.

### 19. Amylose-Extender Sugary Sugary-2

The mature kernel phenotype of the triple mutant *ae su su2* differs from that of any of the component mutants (Table IV). Mature kernel dry weight is similar to that of *ae su;* and sugar and starch concentrations are intermediate between those in *su* and *su su2* and those in *ae, su2, ae su,* and *ae su2* (*205*). Mature and immature kernels contain intermediate levels of WSP (Table VI). This WSP has not been characterized and may or may not be similar to the phytoglycogen accumulating in *su* kernels. Starch from *ae su su2* kernels has been reported to contain 31–54% apparent amylose (Table VII). Starches from *ae su su2* have not

been separated by Sepharose chromatography, and thus the relative sizes of the polysaccharides and degree of branching have not been established.

### 20. Amylose-Extender Sugary Waxy

The mature kernel phenotype of the triple mutant *ae su wx* differs from that of any of the component mutants (Table IV). The dry weight per mature kernel is similar to that of *ae su* and is higher than that of *su*, *ae wx*, and *su wx* (*205*). Quantities of sugars and WSP (Table VI) in mature (*205*) and immature (*204*) kernels are intermediate among the component single and double combinations. Starch content is relatively low, but higher than that of *su* (*205*). The WSP fraction is shown to contain phytoglycogen (Table VI) with characteristics similar to *su* phytoglycogen (*13*). Kernels of *ae su wx* also contain phytoglycogen branching enzyme (*13*).

Starches of *ae su wx* are reported to contain 13–14% apparent amylose when measured by blue-value tests (Table VII), but Yeh and co-workers (*71*) (Table VIII) showed that the apparent amylose is due to the loosely branched nature of the starch polysaccharides. Thus, *wx* blocks amylose accumulation, and *ae* influences the degree of branching. The maximum absorption and extinction coefficient of the polysaccharide–iodine complex is similar to that for *ae wx* (Table VIII). Starch granules isolated from mature *ae su wx* kernels vary from large spherical granules to small aggregated and compound granules (*71*). Most granules are strongly birefringent, but occasional phytoglycogen containing plastids and non-iodine-staining and non-birefringent granule particles are present in the starch granule preparation (*71*).

### 21. Amylose-Extender Sugary-2 Waxy

The *ae su2 wx* kernel phenotype differs from each of the component mutants (Table IV). Mature *ae su2 wx* kernel dry weight is intermediate between that of the lighter *ae* and *ae wx* kernels and the heavier *su2*, *wx*, *ae su2*, and *su2 wx* kernels (*205*). The quantities of sugar in mature (*205*) and immature (*204*) *ae su2 wx* kernels are similar to those in *ae wx*, while WSP and starch concentrations are somewhat higher. The small amount of WSP present (Table VI) has not been characterized, and its similarity to phytoglycogen is unknown. Starch of *ae su2 wx*, based on blue-value tests, has been reported to contain 28% amylose (Table VII). Although not yet determined, this apparent amylose is most likely due to the presence of a loosely branched amylopectin similar to that present in *ae wx* (*69*, *214*).

### 22. Dull Sugary Sugary-2

The mature kernel phenotype of the triple mutant *du su su2* is similar to that of *su* (Table IV). Also, sugar concentrations in mature (*205*) and immature (*204*) *du su su2* kernels are similar to those in *su*, but WSP and starch are higher and

lower, respectively, in *du su su2*. Various amylose percentages have been reported (Table VII). The 77% amylose observed in one study (*224*) is the highest percentage observed in genotypes lacking *ae;* however, because of the low starch content, this genotype has little or no commercial value. As observed with other genotypes, amylose percentage varies with year of production (*224*). Starch granules of *du su su2* are small, similar to *su* (*256*), and exhibit little or no birefringence (*206, 256*). Starch components from *du su su2* have not been separated by Sepharose chromatography, so the precise nature of the polysaccharides is unknown.

### 23. Dull Sugary Waxy

The mature kernel phenotype of *du su wx* is similar to that of *su* (Table IV). The quantity of sugars is similar to that in *su* (*204, 205*). The addition of *du* to *su wx* causes an increase in WSP (Table VI) and a decrease in starch (*205*). The phytoglycogen from *du su wx* has a β-amylolysis limit and chain length similar to that of *su*. The enhanced phytoglycogen accumulation may result from the additive effect of the branching enzymes present in each of the component single mutants (*13*).

Starch from *du su wx* is approximately 100% amylopectin (Table VII) and consists of large and intermediate size polymers (Table VIII). The absorption maximum and extinction coefficient of the amylopectin–iodine complex are similar to those for *wx* and *du wx* amylopectins (Table VIII). Starch granules isolated from mature *du su wx* kernels vary from small spherical to aggregated and compound granules (*71*). Although most granules are strongly birefringent, noniodine staining and nonbirefringent granular particles are also observed in the starch granule preparation (*71*). The granular particles probably are the same as the ultra-fine starch granule fragments reported by Saussy (*26*).

Young kernels of *du su wx* have the major gradient in endosperm development characteristic of normal and the type II minor gradient characteristic of *du* (*26*). However, later in development, much of the central endosperm consists of a noncellular cavity containing starch granules and "phytoglycogen" plastids (*26*). Cells near the pericarp contain amyloplasts with small, compound granules, while more interior cells are filled with large "phytoglycogen" plastids void of starch, which appear unique in that the plastid contents are essentially unstained by iodine (*26*). Since the β-amylolysis limit and mean chain lengths of phytoglycogen from *du su wx* are similar to those for *su* (*13*), the reason for the difference in staining properties of phytoglycogen plastids in *du su wx* and *su* is unknown.

### 24. Dull Sugary-2 Waxy

The mature kernel phenotype of the triple mutant *du su2 wx* differs from that of any of the component mutants (Table IV). Mature kernel dry weight of *du su2*

*wx* kernels is similar to that of the component mutants (*205*). Sugar concentrations in mature (*205*) and immature (*204*) kernels are similar to those in *du wx* kernels. WSP is slightly higher and starch lower in *du su2 wx* compared to *du wx* (Table VI). The WSP has not been characterized, and its similarity to phytoglycogen is unknown. Starch from *du su2 wx* kernels is 100% amylopectin (Table VII), reflecting the effect of *wx*. The BEPT of *du su2 wx* starch granules is low, reflecting the influence of *su2* (*206*).

### 25. Sugary Sugary-2 Waxy

The mature kernel phenotype of the triple mutant *su su2 wx* is similar to that of *su* (Table IV). Kernel dry weight and carbohydrate concentrations in mature (*205*) and immature (*204*) *su su2 wx* kernels are similar to those in *su su2* kernels. The elevated concentration of WSP (Table VI) is assumed to be phytoglycogen, although it has not been characterized. The *wx* gene is epistatic to *su su2*, resulting in the accumulation of starch composed of 100% amylopectin (Table VII). Starch granules show little birefringence (*206*). The BEPT is low, similar to that for *su2* (*206*).

### 26. Amylose-Extender Dull Sugary Waxy

The mature kernel phenotype of the quadruple mutant *ae du su wx* differs from each of the component mutants (Table IV) and varies depending on the sweet corn inbred background (Garwood, unpublished). Mature kernel dry weight is similar to that of *su* kernels (*213*). Starch from *ae du su wx* consists of 100% amylopectin (Table VIII), with most of the polysaccharides of intermediate size (*71*). The degree of branching of the major component (intermediate size) is similar to that of *wx* amylopectin (Table VIII). Aqueously isolated granules contain starch granules with associated nonbirefringent polysaccharides similar to those in *ae du wx*, and extraction of the granule preparation with 10% ethanol removes 27% of the total polysaccharide (*71*). The addition of *su* to *ae du wx* increases the occurrence of small, aggregated, and compound granules (*71*).

Endosperm development in *ae du su wx* is similar to that in *du su wx*, with the type II minor gradient observed and the central endosperm cavity being present by 27 days post-pollination (*26*). Starch granule and phytoglycogen plastid development in *ae du su wx* is similar to that in *su*, except that the quadruple mutant has greater apparent phytoglycogen content at 16 days post-pollination than does *su* or any other mutant combination (*26*). However, with development, there is increasing deterioration of the plastids and central endosperm cells (*26*).

## VIII. Conclusions

By using mutants of maize and other species, progress has been made in understanding the pathways and enzymes involved in starch biosynthesis and the



fine structure of starch
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A number of maiz
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(*96*). It is possible that
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Mutations such as

*Summary of Mutant Effec*

| *Genotype* | *Major* |
|---|---|
| *sh* | ↑ sug |
| *sh2* | ↑ sug |
| *bt2* | ↑ sug |
| *sh4* | ↑ sug |
| *su* | ↑ sugars ↑ phy |
| *wx* | = |
| *ae* | ↑ loos |
| | ↑ app |
| *du* | ↑ app |

[a] Changes relative to *no*
soluble sugars.

fine structure of starch polysaccharides. However, starch biosynthesis and granule formation is still not completely understood. Thus, integration of the information on polysaccharide biosynthesis (Section VI) with that on mutant effects (Section VII) is necessary to evaluate current understanding of polysaccharide biosynthesis and to delineate the limits of this knowledge.

A number of maize endosperm carbohydrate mutants have been shown to influence the *in vitro* activity of particular enzymes (Table X). To date, modification of specific enzyme activities has not been related to endosperm mutants such as *bt* and *su2*. Effects shown in Table X need not necessarily be the primary effect of a mutant, but are the ones known at this writing. Screening for enzyme activities by earlier workers probably would not have detected changes in isozyme activities involving the multiple forms of phosphorylase, starch synthase, and branching enzyme that exist in plants. Thus, more careful examinations will be needed to identify additional enzyme lesions. Also, some mutations may modify the *in vivo* activity of specific enzymes by regulating effector metabolites (*96*). It is possible that current enzyme isolation techniques have not allowed the *in vitro* measurement of certain polysaccharide synthesizing enzymes active in *normal* or mutant tissues. For example, amylosucrase, a bacterial α-D-glucosylase that directly converts sucrose to a glycogen-like α-glucan (*18*), has not been measured in higher plants. Could higher plants have a similar enzyme which is unstable to traditional isolation techniques? Other experimental approaches may be needed to gain information on the precise pathway of starch biosynthesis in the intact, compartmented plant cell.

Mutations such as *sh*, *sh2*, and *bt2* cause major blocks in the conversion of

**Table X**

*Summary of Mutant Effects in Maize Where an Associated Enzyme Lesion Has Been Reported*

| *Genotype* | *Major biochemical changes*[a] | *Enzyme affected* |
|---|---|---|
| *sh* | ↑ sugars ↓ starch | ↓ sucrose synthase |
| *sh2* | ↑ sugars ↓ starch | ↓ ADPG-pyrophosphorylase<br>↑ hexokinase |
| *bt2* | ↑ sugars ↓ starch | ↓ ADPG-pyrophosphorylase |
| *sh4* | ↑ sugars ↓ starch | ↓ pyridoxal phosphate |
| *su* | ↑ sugars ↑ phytoglycogen ↓ starch | ↑ phytoglycogen branching enzyme |
| *wx* | ≃ 100% amylopectin | ↓ starch-bound starch synthase<br>↑ phytoglycogen branching enzyme |
| *ae* | ↑ loosely branched polysaccharide<br>↑ apparent amylose % | ↓ branching enzyme IIb |
| *du* | ↑ apparent amylose % | ↓ starch synthase II<br>↓ branching enzyme IIa<br>↑ phytoglycogen branching enzyme |

[a] Changes relative to *normal*, ↑, ↓ = increase or decrease, respectively. Sugars = the alcohol-soluble sugars.

sucrose to the sugar nucleotides UDPG and ADPG (Table X), indicating the key *in vivo* roles of sucrose synthase and ADPG pyrophosphorylase in starch synthesis. The *su* mutant allows the accumulation of phytoglycogen due to the activity of phytoglycogen branching enzyme (*263–265*). Phytoglycogen branching enzyme activity was also found in *wx* and *du* (*13*), but these mutant kernels did not produce phytoglycogen except when they were incorporated into a sweet corn background (*26*). The double mutant *du wx* contains phytoglycogen branching enzyme and also accumulates phytoglycogen (*13*). Approximately 100% amylopectin is produced in kernels homozygous for *wx* (Table VII). In *wx*, the major starch granule bound starch synthase is missing, but the two soluble starch synthase activities are unaffected (*5*). The *ae* mutant interferes with typical branching causing accumulation of a loosely branched polysaccharide (Table VIII). The presence of this polymer causes an increase in "apparent" amylose percentage when measured by iodine binding methods (Table VII). The branching enzyme IIb, which coelutes with starch synthase I from DEAE cellulose columns, is missing in *ae;* but branching enzymes I and IIa are unaffected (*171*). The *du* mutant causes an increase in apparent amylose content through its effects on starch synthase II (the starch synthase which requires primer) and branching enzyme IIa which coelutes with it from DEAE cellulose columns (*275*).

Interaction of these mutants further clarifies the biosynthetic pathway. For example, the *wx* mutant is epistatic to all other known maize endosperm mutants and no amylose accumulates (Table VII). Mutants such as *sh2*, *bt2*, and *su* cause major reductions in starch accumulation, but in combination with *wx* the starch which is produced is all amylopectin (*208*). In the double mutant *ae wx*, *wx* prevents the production of amylose, and *ae* reduces the degree of branching, resulting in the accumulation of a loosely branched polysaccharide (*69*). The *su* mutant is epistatic to *du*, *su2*, and *wx* relative to accumulation of phytoglycogen; but *ae* and *sh2* are partially epistatic to *su*, causing a marked reduction in the *su* stimulated phytoglycogen accumulation (Table VI). The addition of *du* or *wx* to *ae su* partially overcomes the *ae* inhibitory effect, and phytoglycogen accumulates.

Obviously, our understanding of starch biosynthesis is still incomplete, since mutants occur for which the primary metabolic effect has not been determined. Subsequent evaluation of isozymes and effector compounds and studies of the *in vivo* pattern and rate of $^{14}C$ labeling of intermediates of starch biosynthesis of *normal*, mutants, and mutant combinations should aid in clarifying the nature of the mutations and the pathways of starch biosynthesis. Other aspects of starch formation also remain to be explained. For example, how are starch granules formed? How do reserve starch granules develop species specific shapes? Is a primer needed for starch formation *in vivo?*

In spite of these limitations, the pathway of starch biosynthesis determined using maize mutants can probably be generalized to other plant species because

related mutants have
same enzymes are fo
With the existence of
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extent as maize.

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(*3*) W. Banks and C. T. Edinburgh, 19
(*4*) R. G. Creech, *Adv.*
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(*7*) B. O. Juliano, *in* Cereal Chemis
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(*10*) J. S. Craigie, *in* " Scientific Pub
(*11*) J. F. Frederick, *Ar*
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(*17*) E. J. Hehre, *Adv.*
(*18*) G. Okada and E. J
(*19*) J. D. Dodge, "Th
(*20*) A. R. Archibald, I
(*21*) D. B. Dickinson,
(*22*) E. F. Artschwager
(*23*) L. Lampe, *Bot. G*
(*24*) T. A. Kiesselbach
(*25*) J. C. Shannon, *Ce*
(*26*) L. A. Saussy, M. 1978.
(*27*) C. D. Boyer, R. F
(*28*) E. H. Sanders, *Ce*
(*29*) L. W. Rooney, *i* Chemists, St.

related mutants have occurred in peas, sorghum, barley, and rice and because the same enzymes are found in starch-synthesizing tissues in other plant species. With the existence of isozymes, however, it is possible that the pathway of starch biosynthesis may differ slightly when other species are examined to the same extent as maize.

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# ENZYMES I SYNTHESIS

*Department of Bio*



## I. Intro

Starch, a mixture
energy of the sun, v
serves as a food res
photosynthesizing or
the sun. To utilize st
hydrolysis of the (1—
residues. Enzymes
α-D-(1→4) linkages
bacteria, and animal

Amylases have be
anomeric carbon ato